Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on the Immune System of Laboratory Animals

by J.G. Vos,* J.A. Moore,* and J.G. Zinkl*

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic compounds known. TCDD and other chlorinated dibenzodioxins have been associated with occupational chloracne in workers engaged in the manufacture of technical chlorophenols and their derivatives such as the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (*1*), in lethal liver necrosis in rabbits (*2*), and in the chick edema disease (*3*). More recently TCDD has been shown to be highly teratogenic (*4*).

Laboratory studies have found TCDD to cause severe atrophy of the thymus at sublethal dose levels (*5, 6*). Because the thymus is the central lymphoid organ for cell-mediated immunity and other environmental chemicals have produced immunosuppression (*7*), studies to test the immune response in TCDD-treated laboratory animals were conducted. Methods selected to assess cell-mediated immunity were delayed type hypersensitivity to tuberculin in guinea pigs and rats, and a local graft versus host reaction in mice. The effect of TCDD on the humoral immunity was also studied in guinea pigs.

*National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, Research Triangle Park, North Carolina 27709.

Materials and Methods

Studies on the Effects of TCDD on Humoral and Cell-Mediated Immunity in Guinea Pigs

In each of two experiments, groups of 10 female Hartley strain guinea pigs received 8 weekly doses of 0, 0.008, 0.04, 0.2, or 1.0 μg TCDD/kg body weight. The TCDD in an acetone–corn oil mixture was administered orally in a volume of 1 ml/kg body weight. The animals (mean weight 256 g, ranging between 208 and 301) were housed in groups of five and allowed free access to food and water. Body weights were determined weekly. The guinea pigs were killed with carbon dioxide gas. Heart blood was used for determination of total leukocyte (using a Coulter Counter, Model B) and differential leukocyte counts. The pathologic effects of TCDD, except for the effects on lymphoid organs and skin (second experiment) are reported elsewhere (*6, 8, 9*).

In one experiment, the effect of TCDD on humoral immunity was determined by measuring guinea pig response to a subcutaneous injection of tetanus toxoid. The purogenated toxoid (Lederle Laboratories, Pearl River, New York) was administered in a volume of 0.1 ml into the right hind foot pad at day 28 (1 Lf. tetanus toxoid, aluminum phosphate-adsorbed) and again at day 42 (1 Lf. tetanus toxoid, unadsorbed). Blood was collected (*10*) on days 35 and 49,

and from the heart at the end of the experiment. The serum tetanus-antitoxin concentrations were determined by using a modified single radial immunodiffusion technique (*11*); the agar contained appropriate dilutions of tetanus toxoid and the serum (10 μl) was allowed to diffuse from the well.

The effect of TCDD on cell-mediated immunity was studied in a second experiment by measuring the delayed-type hypersensitivity to tuberculin. Guinea pigs were sensitized by a subcutaneous injection (0.05 ml) of an oil suspension containing killed *Mycobacterium tuberculosis* $H_{37}Ra$ (complete H_{37} Ra adjuvant) (Difco Laboratories, Detroit, Michigan) in the hind foot pad at day 35. The delayed hypersensitivity was tested by intradermal tuberculin injections [1.25 μg tuberculin PPD (Parke-Davis, Detroit, Michigan) in 0.1 ml diluent] on days 47 and 54. The diameter (mm) of the skin reactions was determined 24 and 48 hr after the tuberculinations. In addition, the thickness of the reaction was determined by measuring the thickness of the tuberculin reaction and of the normal skin. Subtraction of the latter from the former value gave the thickness of the tuberculin reaction. To evaluate a possible indirect immunosuppressive effect by adrenocortical hyperfunction, pooled serum samples were analyzed for cortisol and corticosteron levels by using the double isotope derivative method (*12*) (New England Nuclear, Boston, Massachusetts).

TCDD Effect on Cell-Mediated Immunity in Rats

Groups of 10 random bred female albino rats (CD stock, mean weight 185 g, ranging from 165 to 201) were given oral doses, weekly for 6 weeks, of 0, 0.2, 1.0, or 5.0 μg TCDD/kg body weight. A skin test, similar to that in guinea pigs, was performed by injecting 0.05 ml oil suspension of killed *Mycobacterium tuberculosis* in the right hind foot pad at day 28 and by injecting 5 μg tuberculin PPD in 0.1 ml diluent into the skin of the shaved flank at day 42. The diameter and thickness of the reactions were measured after 24 and 48 hr. Half of the animals of each group were euthanatized and necropsied at day 45. The weight of lymphoid organs and adrenals was determined as were total and differential leukocyte counts. Tissues, including the skin at the site of the tuberculin injection, were processed for histology. Liver pathology is published elsewhere (*8*).

Effect of TCDD on Cell-Mediated Immunity in Mice.

In this experiment, the graft versus host activity of donor (C57B1/6) spleen cells was measured by injecting them into the right hind foot pad of hybrid recipient mice, $B_6D_2F_1$, obtained by mating the parental strains C57B1/6 and DBA-2 which differ from each other at the major histocompatibility [H-2] locus. In this situation, donor cells are tolerated by the hybrid because the graft does not possess any antigens which are foreign to the host, but the donor cells react against DBA-2 antigen of host cells. The weight of the enlarged injected lymph node, which is due to the immunological reaction, compared with the weight of the uninjected left popliteal nodes (right/left ratio) was the parameter used for the graft versus host activity of the donor spleen cells (J. A. M. Kerckhaert, personal communication, 1972).

Groups of five to seven male, 2-month-old donor mice (housed individually, mean weight 24.4 g, ranging between 22.0 and 27.2 g) were orally dosed weekly with 0, 0.2, 1.0, 5.0, or 25 μg TCDD/kg body weight (in a volume of 0.1 ml per 20 g body weight) for 4 weeks. After 4 weeks, the animals were killed with carbon dioxide gas. Thymuses were weighed and processed for histology. Cell suspensions were made from the pooled spleens of each donor group. Spleens were minced with scissors and the fragments were gently pressed through a nylon gauze (200 μ pore diameter). The cells were washed with a MEM (Eagle) suspension culture medium (Flow Laboratories, Rockville, Maryland) containing 100 units of penicillin and 100 μg streptomycin per milliliter. Cell suspensions were counted with a Coulter Counter. Viability was determined with ery-

throsin B (0.4% in PBS). Final cell suspensions contained 2×10^8 viable nucleated cells ml medium. These donor cells (0.05 ml, 1×10^7 viable cells) were injected subcutaneously by using a 0.25 ml syringe with a 25-gauge needle into the right hind foot pad of 10–12-week-old male recipient animals. The recipient mice were killed after 7 days. Right (injected) and left (uninjected) popliteal lymph nodes were removed, trimmed free from adipose tissue, and weighed to an accuracy of 0.05 mg. The right/left ratio was determined. In a preliminary study, 10 control lymph nodes weighed 0.495 ± 0.185 mg (SD) and the lymph node weights of 10 recipients injected with 5×10^6 viable

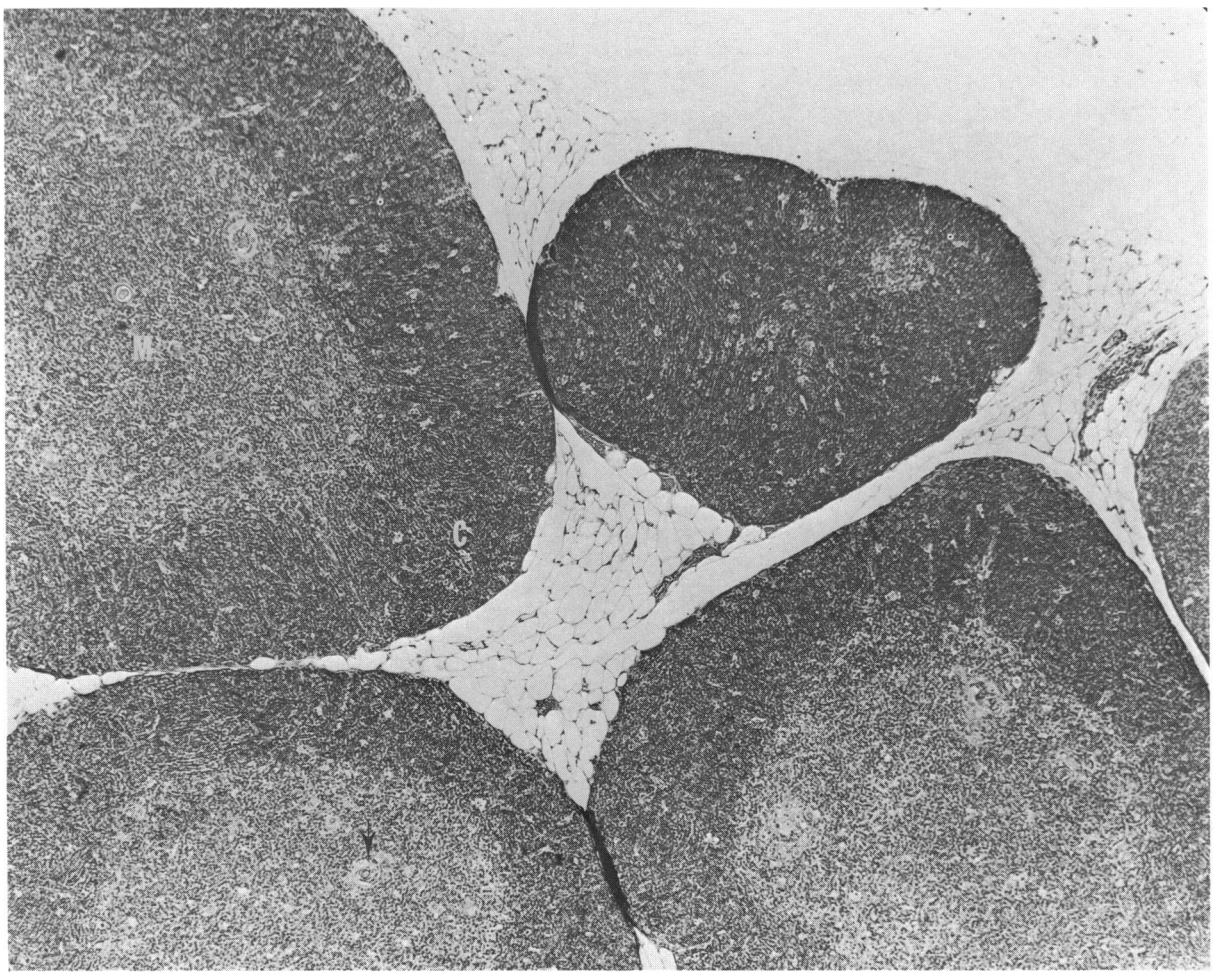

FIGURE 1. Thymus of a control guinea pig from the skin test experiment. Note the densely packed lymphocytes in the cortex (C) and the Hassall bodies (arrow) in the medulla (M). Hematoxylin and eosin; 61 ×.

syngeneic F_1 hybrid cells were 0.495 mg ± 0.136, thus demonstrating that lymph node enlargement after 7 days is not due to a drainage of cells but to a graft versus host reaction. Recipients injected with C57-B1/6 cells showed a massive proliferation of lymphoid cells on microscopic examination of the lymph nodes.

The data obtained in the four experiments are presented as mean values and standard deviations. Dunnett's multiple comparisons test (*13*) was used to make treatment con-

FIGURE 2. Thymus of guinea pig that was killed when moribund on day 27 after receiving four weekly oral doses of 1 μg TCDD/kg. A severe cortex atrophy (C) can be seen, with destruction of lymphocytes that are phagocytized by macrophages ("starry-sky" appearance). Large cystic Hassall bodies (arrow) filled with polymorphonuclear leukocytes are present in the medulla (M). Hematoxylin and eosin; 61 ×.

trol comparisons, usually two-sided, except for skin reaction variables (one-sided). In addition, a nonparametric test (Jonckheere's test) (*14*) was used to test for monotonic dose–response relationships. In the case of organ weights, only the organ/body weight ratios were tested.

Results

Experiments with Guinea Pigs

All guinea pigs treated with TCDD at the 1 μg/kg level died or were killed when moribund between 24 and 32 days (mean 28 days). They showed severe weight loss (*6*), lymphopenia (9), and depletion of the lymphoid organs, especially the thymus (*8*). Microscopically (Figs. 1 and 2), there was a severe atrophy of the thymic cortex with considerable destruction of lymphocytes, the nuclear debris being engulfed by macrophages ("starry sky"). Large cystic Hassall bodies, filled with polymorphonuclear leukocytes, were seen in the medulla.

All animals at the 0, 0.008, 0.04, and 0.2 μg/kg levels survived in both experiments. Body weights, organ weights and leukocyte counts are given in Tables 1 and 2. Weight gain was significantly lower in both 0.2 μg/kg groups. Absolute thymus weight was significantly reduced at the 0.04 (experiment 1) and 0.2 μg/kg (experiments 1 and 2) dose levels, but the relative (organ to body weight ratio) weights only at the 0.2 μg/kg levels. The absolute weights of the superficial cervical lymph nodes (experiment 1) were significantly decreased in the 0.2 μg/kg group. At this level, the relative adrenal weights were significantly increased in both studies. Total leukocyte values were significantly decreased in the 0.04 μg/kg group of experiment 1 and in the 0.2 μg/kg group of ex-

Table 1. Body weights, organ weights, and leukocyte counts of guinea pigs treated with TCDD for 8 weeks (tetanus toxid stimulation).[a]

	Weekly TCDD dose				Dose-response test
	0	0.008 μg/kg	0.04 μg/kg	0.2 μg/kg	
Final body weight, g	580.2 ± 34.3	597.2 ± 37.5	551.9 ± 48.9	497.1 ± 37.8[b]	$P < 0.01$
Organ weights, mg					
Thymus	901 ± 246	741 ± 163	672 ± 161[c]	476 ± 70[b]	
Spleen	1036 ± 280	858 ± 144	870 ± 174	793 ± 133	
Cervical lymph nodes	224 ± 35	202 ± 33	199 ± 38	179 ± 32[c]	
Right popliteal lymph node	33.4 ± 13.7	36.8 ± 11.8	38.9 ± 12.4	30.5 ± 12.4	
Adrenals	294 ± 33	294 ± 42	302 ± 38	285 ± 36	
Organ/body weight ratio × 10^3					
Thymus	1.54 ± 0.39	1.24 ± 0.27	1.21 ± 0.24	0.96 ± 0.14[b]	$P < 0.01$
Spleen	1.79 ± 0.50	1.44 ± 0.22	1.58 ± 0.30	1.59 ± 0.23	NS
Cervical lymph nodes	0.386 ± 0.055	0.338 ± 0.053	0.361 ± 0.064	0.362 ± 0.073	NS
Right popliteal lymph node	0.0584 ± 0.0257	0.0612 ± 0.0182	0.0704 ± 0.0201	0.0613 ± 0.0268	NS
Adrenals	0.507 ± 0.047	0.490 ± 0.049	0.548 ± 0.060	0.595 ± 0.064[c]	$P < 0.01$
Total leukocytes × 10^3 per mm^3	6.41 ± 1.88	5.05 ± 1.16	4.85 ± 1.24[c]	4.91 ± 0.99	$P < 0.05$
Lymphocytes × 10^3 per mm^3	4.16 ± 1.47	2.39 ± 0.73[b]	2.87 ± 1.03[c]	2.59 ± 0.46[c]	$P < 0.05$

[a] Mean values ± SD, 10 animals per group except at the 0.2 μg/kg level (7 animals).
[b] $P < 0.01$.
[c] $P < 0.05$.

periment 2. Significantly decreased lymphocyte counts were found in experiment 1 at all 3 dose levels. Significant monotonic dose–response relationships were determined for body weights (decrease), relative thymus weights (decrease), relative adrenal weights (increase), and total leukocyte and lymphocyte counts (decrease). Serum cortisol and corticosteron values that were measured only in experiment 2 (Table 2) were the same in all four groups. At microscopic examination of the lymphoid organs and adrenals, no effects were seen except for slight cortical atrophy of the thymus at the 0.2 μg/kg level.

Tetanus Toxoid Stimulation: Guinea Pigs

Serum tetanus antitoxin concentrations are given in Table 3. Seven days after the first tetanus toxoid injection (day 35) there was a small but significant increase at the 0.008 and 0.04 μg/kg levels. Serum antitoxin values were significantly decreased at the 0.2 μg/kg level in the secondary response (days 49 and 56), but there was no significant effect at the 0.008 and 0.04 μg/kg dose levels. Weights and histology of the injected right popliteal lymph nodes were the same in all four groups.

Skin Test: Guinea Pigs

The diameter and thickness of the skin reactions, measured 24 and 48 hr after tuberculination are given in Table 4. The diameter of the skin reactions in the 0.04 μg/kg group were significantly reduced when measured 48 hr after the first, and 24 and 48 hr after the second intradermal tuberculin injection. At the 0.2 μg/kg level, all values (diameter and thickness skin reaction) were significantly decreased. The dose response test showed also a highly significant decrease of all data. Microscopically (Fig. 3*a*, 3*b*),

Table 2. Body weights, organ weights, leukocyte counts, and serum corticosteroid levels of guinea pigs treated with TCDD for 8 weeks (skin test).[a]

	Weekly TCDD dose				Dose-response test
	0	0.008 μg/kg	0.04 μg/kg	0.2 μg/kg	
Final body weight, g	572.9 ± 64.5	573.3 ± 64.2	529.6 ± 61.4	473.1 ± 41.6[b]	$P < 0.01$
Organ weights, mg					
Thymus	760 ± 208	819 ± 216	649 ± 133	409 ± 139[b]	
Right popliteal lymph node	163 ± 99	130 ± 33	90 ± 61	82 ± 25	
Adrenals	290 ± 42	295 ± 36	267 ± 41	286 ± 28	
Organ/body weight ratios × 10^3					
Thymus	1.32 ± 0.30	1.43 ± 0.31	1.23 ± 0.22	0.86 ± 0.28	$P < 0.01$
Right popliteal lymph node	0.278 ± 0.141	0.229 ± 0.059	0.171 ± 0.114	0.176 ± 0.059	$P < 0.05$
Adrenals	0.506 ± 0.044	0.519 ± 0.070	0.505 ± 0.064	0.612 ± 0.102[c]	$P < 0.05$
Total leukocytes × 10^3 per mm^3	8.84 ± 4.21	8.23 ± 2.48	7.26 ± 1.66	6.14 ± 2.91[c]	$P < 0.01$
Lymphocytes × 10^3 per mm^3	4.27 ± 2.29	4.23 ± 1.31	3.72 ± 1.17	3.63 ± 2.37	$P < 0.05$
Corticosteroids (pooled samples), μg/100 ml					
Cortisol	93.3	92.5	85.2	88.2	
Corticosteron	1.4	1.2	1.5	1.3	

[a] Mean values ± SD, 10 animals per group.
[b] $P < 0.01$.
[c] $P < 0.05$.

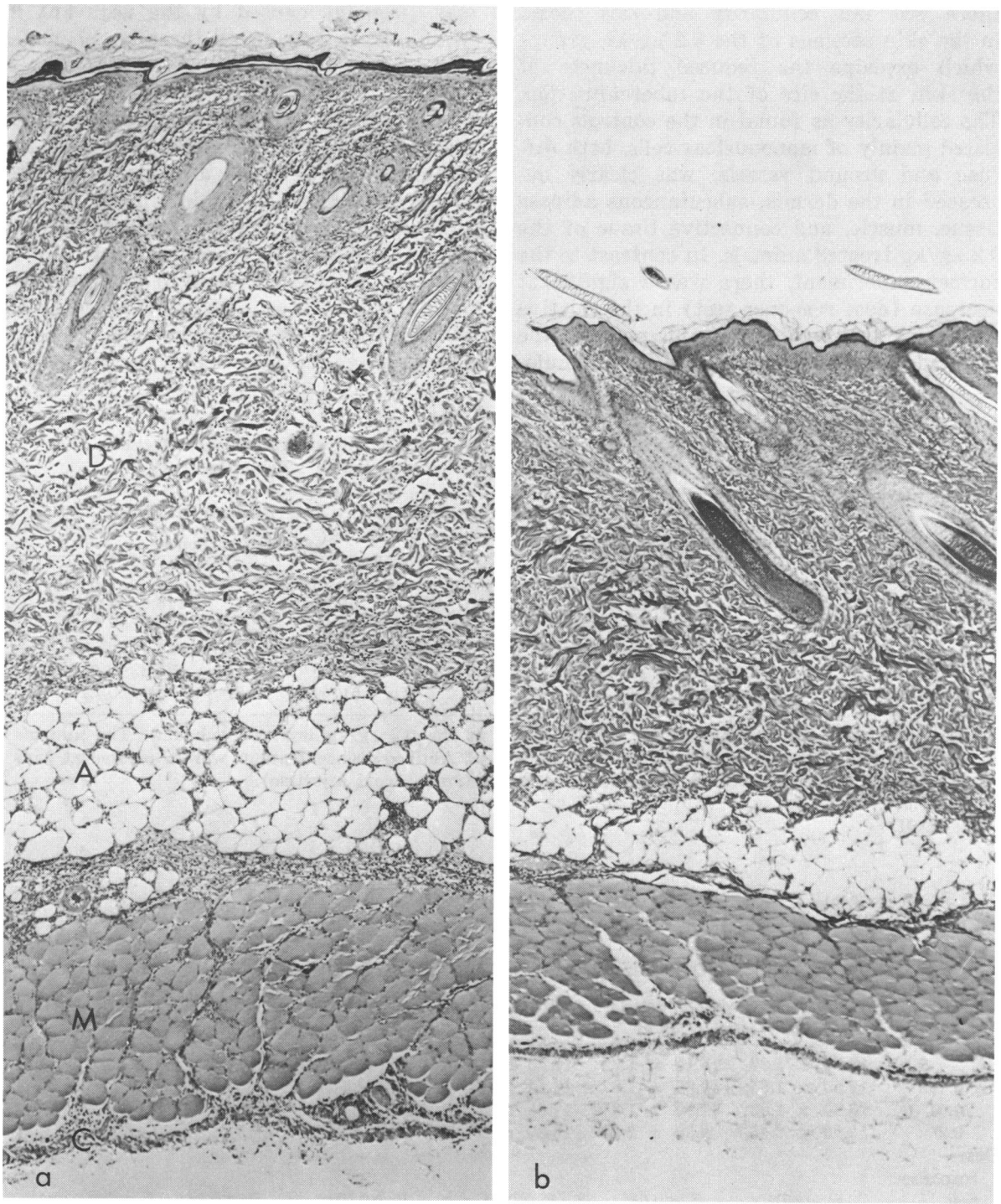

FIGURE 3. Forty-eight hour skin reaction to 1.25 μg tuberculin PPD in guinea pigs sensitized 17 days earlier with 0.05 ml of an oil suspension containing killed *Mycobacterium tuberculosis* H_{37} *Ra:* (*a*) control animal, note the edema and diffuse cellularity of the dermis (D) and the infiltration both diffuse and around small vessels in the subcutaneous adipose tissue (A), muscle (M) and connective tissue (C); (*b*) guinea pig treated with 8 weekly doses of 0.2 μg TCDD/kg. There is less edema and less cellularity in the dermis and the focal cellular infiltration is much smaller. Hematoxylin and eosin; 52 ×.

there was less cellularity and less edema in the skin sections of the 0.2 μg/kg groups which explains the reduced thickness of the skin at the site of the tuberculination. The cellularity as found in the controls consisted mainly of mononuclear cells, both diffuse and around vessels, was clearly decreased in the dermis, subcutaneous adipose tissue, muscle, and connective tissue of the 0.2 μg/kg treated animals. In contrast to the former experiment, there was a significant decrease (dose-response test) in the relative weight of the popliteal lymph node of the injected right hind leg (Table 2). Granulomas, probably caused by the adjuvant injection, were seen in all these lymph nodes.

Experiment with Rats

All animals survived the experimental period. Body weights, organ weights, and leukocyte counts of the animals killed after 45 days are given in Table 5. Body weights and absolute and relative thymus weights were significantly reduced at the 5.0 μg/kg level. A significant increase in relative spleen weight is also seen at that dose level. The effect of TCDD on adrenal weight in the rat differed from the response in the guinea pig.

Table 3. Serum antitoxin values as measured by single radial immunodiffusion of guinea pigs treated with TCDD for 8 weeks (tetanus toxoid stimulation).[a]

Weekly dose of TCDD, μg/kg	Tetanus antitoxin concentrations, International Units/ml		
	Primary response (day 35)	Secondary response	
		Day 49	Day 56
0	5.55 ± 0.69	259.7 ± 99.7	246.0 ± 92.8
0.008	6.42 ± 0.64[b]	225.4 ± 98.5	265.4 ± 79.9
0.04	6.71 ± 0.61[c]	203.2 ± 57.7	230.0 ± 51.6
0.2	5.29 ± 0.82	149.2 ± 32.8[c]	175.8 ± 47.8[b]
Dose-response test	NS	$P < 0.01$	$P = 0.05$

[a] Mean values ± SD, 10 animals per group, except at the 0.2 μg/kg level (7 animals). The animals were injected with tetanus toxoid at days 28 and 42. Antibody concentrations were measured at days 35, 49, and 56 (7, 21, and 28 days after the first tetanus toxoid injection).
[b] $P < 0.05$.
[c] $P < 0.01$.

Table 4. Skin reaction (delayed hypersensitivity to tuberculin) of guinea pigs treated with TCDD for 8 weeks.[a]

Weekly dose of TCDD, μg/kg	Diameter skin reaction, mm				Thickness skin reaction, mm (tuberculination on day 54)	
	Tuberculineation on day 47		Tuberculination on day 54			
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
0	18.20 ± 1.87	15.15 ± 1.72	19.45 ± 1.23	15.20 ± 1.90	2.23 ± 0.49	1.73 ± 0.43
0.008	17.50 ± 1.35	13.85 ± 1.67	18.45 ± 1.23	13.85 ± 1.73	2.10 ± 0.60	2.00 ± 0.54
0.04	16.80 ± 1.83	12.90 ± 1.31[b]	17.65 ± 0.91[c]	13.40 ± 1.13[b]	1.93 ± 0.46	1.38 ± 0.34
0.2	12.90 ± 2.56[c]	8.80 ± 2.98[c]	15.20 ± 2.91[c]	8.95 ± 3.25[c]	1.13 ± 0.43[c]	0.63 ± 0.41[c]
Dose-response test	$P < 0.01$	$P < 0.01$.	$P < 0.01$.	$P < 0.01$.	$P < 0.01$.	$P < 0.01$

[a] Mean values ± SD, 10 animals per group. The animals were sensitized on day 35 with 0.05 ml of an oil suspension containing killed *Mycobacterium tuberculosis*. Intradermal tuberculination (1.25 μg tuberculin PPD) was performed on days 47 and 54. Skin reactions were measured 24 and 48 hr after tuberculination.
[b] $P < 0.05$.
[c] $P < 0.01$.

Absolute adrenal weights decreased significantly in the rat at the 1.0 and 5.0 μg/kg dose levels. The dose-response test for the decrease in relative adrenal weights was highly significant. Also, the total leukocyte and lymphocyte counts in the rat differed from the response in the guinea pig. There was no lymphopenia, but some increase of both cell counts, showing a slight but not significant dose-response pattern. Microscopically, the only effect seen in the lymphoid organs and adrenals, was a slight to moderate cortical atrophy in the thymuses of the 5 μg/kg group. Also, the cellularity of the cortex was somewhat less dense. The results of the measurements of the skin reactions are given in Table 6. Also in contrast with the guinea pig, there was no effect on the

Table 5. Body weights, organ weights, and leukocyte counts of rats treated with TCDD for 6 weeks (skin test).[a]

	Weekly TCDD dose				Dose-response test
	0	0.2 μg/kg	1.0 μg/kg	5.0 μg/kg	
Final body weight, g	261.6 ± 28.2	264.0 ± 17.4	263.6 ± 25.6	225.0 ± 17.1[b]	$P < 0.05$
Organ weights, mg					
Thymus	318 ± 112	285 ± 50	289 ± 16	132 ± 39[b]	
Spleen	507 ± 85	498 ± 95	536 ± 53	564 ± 79	
Cervical lymph nodes	43.0 ± 16.1	40.0 ± 10.3	37.6 ± 7.6	37.4 ± 7.3	
Adrenals	80.6 ± 10.6	71.2 ± 7.2	61.2 ± 11.2[b]	55.0 ± 13.8[c]	
Organ/body weight ratios × 10^3					
Thymus	1.20 ± 0.32	1.09 ± 0.27	1.10 ± 0.13	0.59 ± 0.16[c]	$P < 0.05$
Spleen	1.94 ± 0.24	1.89 ± 0.35	2.04 ± 0.15	2.53 ± 0.48[c]	$P < 0.05$
Cervical lymph nodes	0.163 ± 0.049	0.153 ± 0.043	0.142 ± 0.016	0.167 ± 0.032	
Adrenals	0.312 ± 0.059	0.269 ± 0.014	0.231 ± 0.028	0.242 ± 0.044	$P < 0.01$
Total leukocytes × 10^3, per mm^3	5.88 ± 2.35	7.98 ± 4.41	7.68 ± 2.32	10.65 ± 5.00	NS
Lymphocytes × 10^3, per mm^3	4.13 ± 1.63	5.94 ± 2.80	5.53 ± 1.89	7.65 ± 3.11	NS

[a] Mean values ± SD, five animals per group.
[b] $P < 0.05$.
[c] $P < 0.01$.

Table 6. Skin reaction (delayed hypersensitivity to tuberculin) of rats treated with TCDD for 6 weeks.[a]

Weekly dose of TCDD, μg/kg	Diameter skin reaction, mm		Thickness skin reaction, mm	
	24 hr	48 hr	24 hr	48 hr
0	14.65 ± 2.94	10.95 ± 2.53	2.10 ± 0.45	1.46 ± 0.30
0.2	15.15 ± 2.49	9.80 ± 2.74	1.90 ± 0.59	1.42 ± 0.44
1.0	15.40 ± 1.93	10.30 ± 2.52	2.23 ± 0.68	1.58 ± 0.64
5.0	14.75 ± 1.83	10.45 ± 2.99	1.88 ± 0.70	1.65 ± 0.54

[a] Mean values ± SD, 10 animals per group. The animals were sensitized on day 28 with 0.05 ml of an oil suspension containing killed *Mycobacterium tuberculosis*. Intradermal tuberculination (5 μg tuberculin PPD) was performed on day 42. Skin reactions were measured 24 and 48 hr after tuberculination.

diameter the thickness, or histological appearance of the tuberculin reactions in the rats.

Experiment with Mice

One animal of the 25 μg/kg group died after 24 days. Body and thymus weights of the donor mice are given in Table 7. There was no difference in the final body weights of controls and TCDD-treated animals due to lower initial weights of the controls, but there was a significant effect on weight gain at the 25 μg/kg level. Absolute and relative thymus weights were signficantly reduced at the 5.0 and 25 μg/kg dose levels. Mean thymus weight in the high dose group was only 13% of the mean weight of the controls. Microscopically (Figs. 4 and 5), there

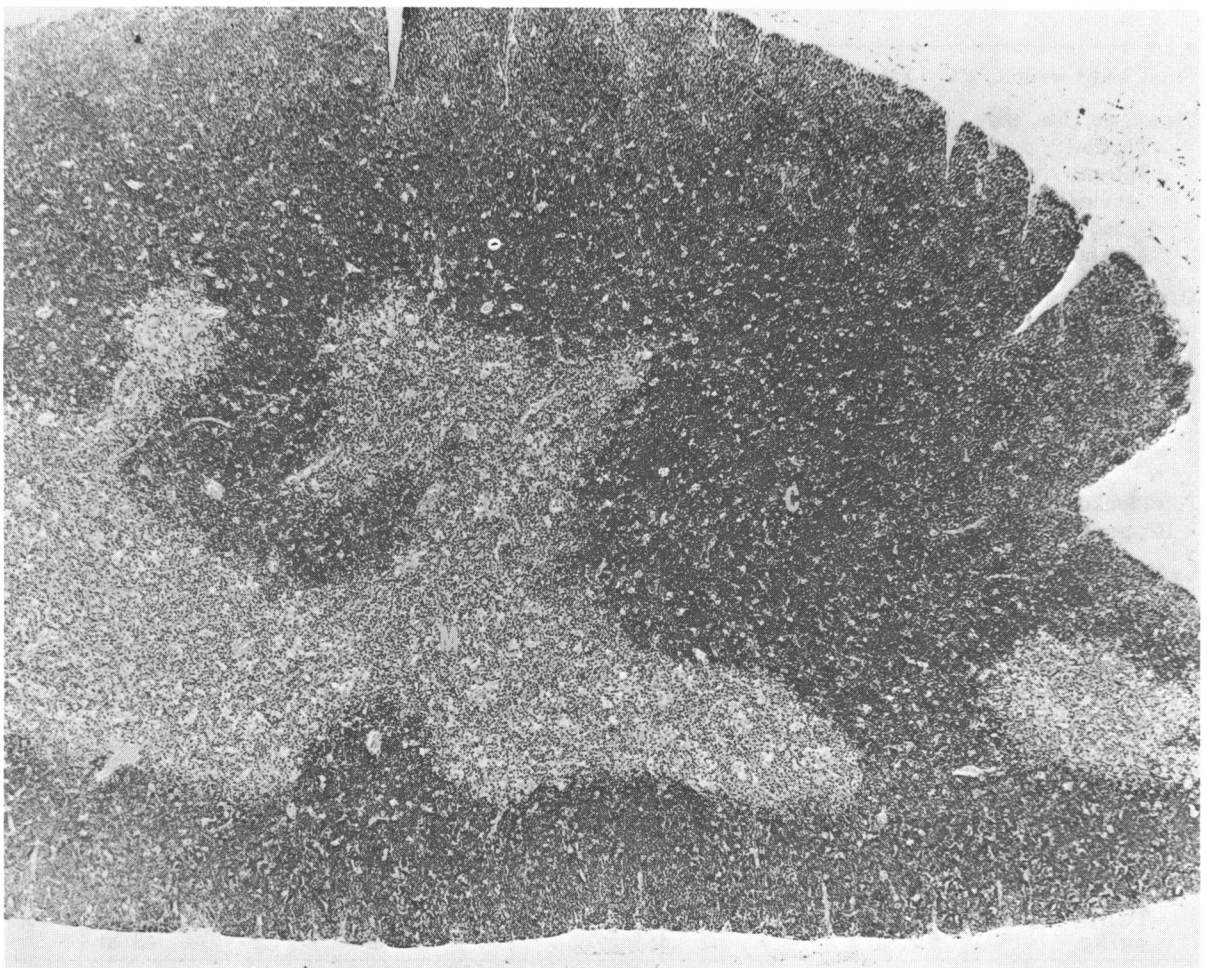

FIGURE 4. Thymus of a control mouse with the cortex at C and the medulla at M. Hematoxylin and eosin; × 61.

was a nearly complete loss of the thymic cortex. The cellularity of the remaining cortex was less dense and there was destruction of lymphocytes. Spleens of the animals receiving 25 μg/kg TCDD were very small. The yield of spleen cells was too small to inject a sufficient number of recipients. As shown in Table 8, injection of 1×10^7 viable nucleated spleen cells from the 5 μg/kg group resulted in a highly significant decreased graft versus host activity when injected into the hind foot pad of the recipients ($B_6D_2F_1$). The mean popliteal lymph node ratio in the recipients injected with cells from the 1

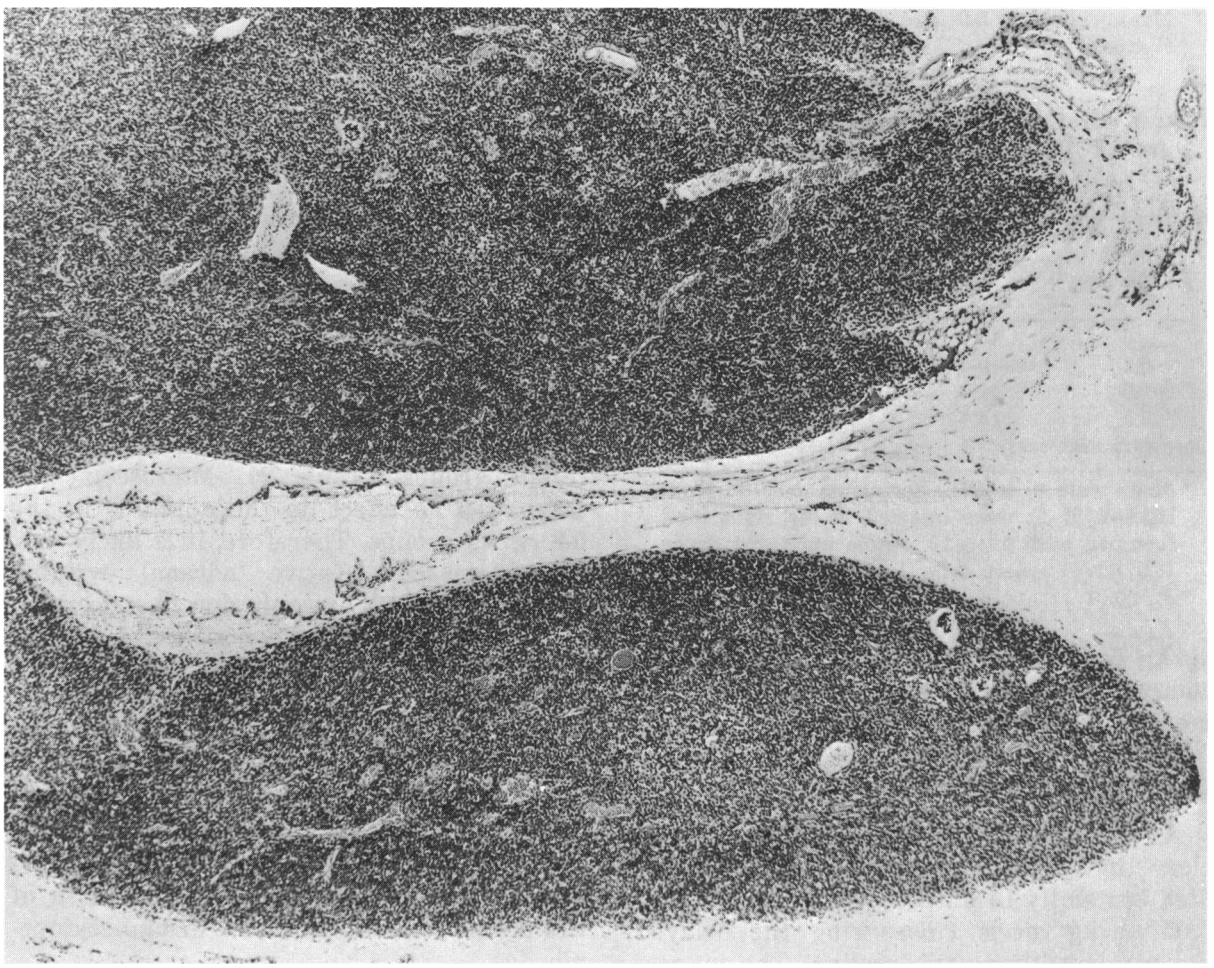

FIGURE 5. Thymus of a mouse that was killed after receiving four weekly oral doses of 25 μg TCDD/kg. Severe cortex atrophy is present; the cellularity in the remaining cortex is less dense making a distinction between cortex and medulla difficult at this magnification. Hematoxylin and eosin; 61 $\times$.

Table 7. Body and thymus weights of donor mice treated with TCDD for 4 weeks.[a]

Weekly dose of TCDD, μg/kg	Final body weight, g	Weight change, g	Thymus, mg	Thymus/body weight ratio × 10^3
0	26.50 ± 0.29	+2.24 ± 0.42	51.8 ± 4.1	1.95 ± 0.14
0.2	25.84 ± 2.80	+1.26 ± 2.09	41.2 ± 14.6	1.57 ± 0.46
1.0	26.34 ± 2.38	+1.78 ± 1.27	40.8 ± 10.0	1.56 ± 0.33
5.0	25.76 ± 2.10	+1.04 ± 2.13	29.0 ± 5.8[b]	1.12 ± 0.15[b]
25	22.02 ± 2.44	−1.92 ± 2.69[b]	6.7 ± 9.1[b]	0.31 ± 0.09[b]
Dose-response test	NS			P <0.01

[a] Mean values ± SD, five or six animals per group.
[b] P <0.01.

Table 8. Graft versus host activity of spleen cells from TCDD-treated donor mice injected into recipients.[a]

Donor TCDD dose, μg/kg	Recipients	
	No.	Right/left ratio Popliteal lymph nodes
0	10	5.83 ± 4.32
0.2	9	5.82 ± 2.47
1.0	12	3.62 ± 1.47
5.0	14	1.98 ± 0.72[b]
Dose-response test		P <0.01

[a] Mean values ± SD. Recipients (C57Bl/6 × DBA–2, F–1) were injected in the right hind foot pad with 1 × 10^7 viable nucleated donor (C57Bl/6) spleen cells.
[b] P <0.01.

μg/kg donor group was 38% lower than the control value. There was a highly significant monotonic dose-response relationship.

Discussion

As shown in Table 4 and Figure 3, it is clear that TCDD suppressed the cell-mediated immunity in guinea pigs at the 0.2 and 0.04 μg/kg levels (measuring the delayed hypersensitivity to tuberculin). The vulnerability of the lymphopoietic system, having a high mitotic activity, is clearly demonstrated by the thymus atrophy and lymphopenia (Tables 1 and 2, Figs. 1 and 2). In this context, it is worth mentioning that inhibition of mitosis has been observed in dividing endosperm cells of the African blood lily when exposed to TCDD (*15*). Also, there was a significant reduction in the relative weights of the stimulated popliteal lymph nodes in the skin test experiment (Table 2). Humoral immunity, measuring the antibody production against tetanus toxoid, was slightly depressed in the guinea pig at the 0.2 μg/kg level (Table 3). There was no effect on the weights of the stimulated popliteal lymph nodes in this experiment (Table 1).

In the second guinea pig experiment, there was no difference in serum cortisol and corticosteron concentrations between the different groups (Table 2). Microscopically, there was no effect on the adrenals in the 0.2 μg/kg groups. Therefore, it is likely that the increased relative adrenal weights (Tables 1 and 2) are only due to a decrease in body weight gain and not due to adrenocortical hyperfunction. Thus, indirect immunosuppression by stimulation of adrenocortical activity can be excluded. Besides the effect on the lymphoid system, there was only minor pathology in guinea pigs receiving a lethal dose of TCDD, i.e., mild liver injury, hyperplasia of the bladder epithelium, hemorrhages, atrophy of the zona glomerulosa of the adrenal cortex (*8*) and thrombocytopenia (*9*).

Analogous to the situation in the guinea pig, there was suppression of the cell-mediated immunity in the mouse. The graft versus host activity of donor spleen cells was significantly suppressed at the 5.0 μg/kg level. Donor cells of the 1 μg/kg group gave a graft versus host response that was 62% of the control value (Table 8). Thymus atrophy (Table 7, Figs. 4 and 5) and lymphopenia

(*9*) were sensitive indices for TCDD exposure. As in the case of the guinea pig, liver pathology was mild (*8*).

In contrast to the guinea pig and mouse study, there is quite a different situation in the rat. Cell-mediated immunity (delayed hypersensitivity to tuberculin) was not suppressed (Table 6). Possibly, this test is not sensitive enough to detect an immunosuppressive effect in rats. Also, except for thymic atrophy at sublethal dose levels, there was no lymphopenia (Table 5). In addition, there was a dose-related decrease in both absolute and relative adrenal weight (Table 5), indicating an adrenal hypofunction. However, in view of the marked induction by TCDD of glucuronyl transferase in rat liver (*16*), an important enzyme involved in the metabolism of corticosteroids, adrenocortical hyperfunction regulated through the hypothalamus-pituitary-adrenocortical axis would be expected. An inhibitory effect on adrenal hypertrophy, induced by surgical trauma, was seen in rats treated with PCB (unpublished data) and with *p,p'*-DDT; in this latter study interference with the feedback mechanism of glucocorticoid hormones homeostasis was proposed (*17*).

In rats, exposed to lethal concentrations of TCDD, there was severe liver injury (degenerative and necrotic changes), thyroid pathology, hemorrhages (*8*), platelet depression, increased serum bilirubin values, and increased SGOT and SGPT activities (*9*). Liver damage can be considered to be the major cause of death in rats exposed to TCDD. In both guinea pigs and mice, there was only mild liver injury (*8*). The most significant findings are seen in the lymphoid system. Suppression of the cell-mediated immunity might be the major cause of death in these species. To test this hypothesis, a study is indicated to determine whether lymphoid (thymus) cell grafts are capable to protect mice from dying when exposed to concentrations of TCDD that are lethal for the control mice. Also, it would be worthwhile determining whether there is a difference in lethal TCDD levels in mice kept under sterile and under conventional or SPF conditions. Experiments will be conducted to determine the effect of *in utero* exposure of TCDD upon the cell-mediated immune reponse of the offspring.

Summary

In two experiments, groups of 10 female guinea pigs were dosed weekly for 8 weeks with 0, 0.008, 0.04, 0.2, or 1.0 μg/kg TCDD/kg body weight to test the cell-mediated and humoral immunity. All animals at the 1 μg/kg level died or were killed when moribund, they showed severe weight loss, lymphopenia,, and atrophy of the lymphoid organs. Weight gain was depressed at the 0.2 μg/kg level. Cell-mediated immunity was assessed by vaccination of the remaining animals with an oil suspension of killed *Mycobacterium tuberculosis*. Thymus atrophy and lymphopenia was observed. The diameters of the skin reactions, measured 24 and 48 hr after tuberculination, were significantly reduced at the 0.2 and 0.04 μg/kg levels. Indirect immunosuppression by stimulation of adrenocortical activity was excluded. The humoral immune system was stimulated in a second experiment by tetanus toxoid injections at days 28 and 42. Serum tetanus antitoxin concentrations were slightly depressed in the 0.2 μg/kg level at days 49 and 56.

A skin test, similar to that in guinea pigs, was done in rats treated weekly for 6 weeks with 0, 0.2, 1.0, and 5.0 μg TCDD/kg body weight. Animals at the 5 μg/kg level had significant lower body, thymus and adrenal weights. No effect was found on the skin reactions.

Cell-mediated immunity was tested in mice in a graft versus host assay. Groups of donor mice (C57B1/6) were treated weekly for 4 weeks with 0, 0.2, 1.0, 5.0, and 25 μg TCDD/kg body weight. Weight gain was depressed at the 25 μg/kg level. There was a remarkable thymic atrophy. Parental strain spleen cells (up to the 5.0 μg/kg group) were injected into the feet of hybrid recipients (C57B1/6 × DBA-2 F-1). The weights of the draining popliteal lymph node, as a parameter for the graft versus host activity, were significantly lower in the animals injected

with spleen cells from the 5 μg/kg donor group.

It is concluded that TCDD at sublethal dose levels suppresses the cell-mediated immunity in both guinea pigs and mice. Humoral immunity was slightly suppressed in the guinea pig. The possible role of immune suppression in the death of TCDD-treated guinea pigs and mice is discussed in view of the absence of major pathologic effects except in the lymphoid system.

Acknowledgements

The authors are grateful to Mrs. L. D. Lawson for valuable technical assistance and to Dr. J. K. Haseman for statistical analyses.

REFERENCES

1. Kimmig, J., und Schulz, K. H. Berufliche Akne (Sog. Chlorakne) durch chlorierte aromatische zyklische Äther. Dermatologica 115: 540 (1957).
2. Bauer, H., Schulz, K. H., und Spiegelberg, U. Berufliche Vergiftungen bei der Herstellung von Chlorphenol—Verbindungen. Arch. Gewerbepath. Gewerbehyg. 18: 538 (1961).
3. Higginbotham, G. R., et al. Chemical and toxicological evaluations of isolated and synthetic chloro derivatives of dibenzo-*p*-dioxin. Nature 220: 702 (1968).
4. Courtney, K. D., and Moore, J. A. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 20: 396 (1971).
5. Buu-Hoï, N. P., et al. Organisms as targets of "dioxin" (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) intoxication. Naturwiss. 59: 174 (1972).
6. Harris, M., Moore, J. A., and Vos, J. G. General biological effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. No. 5: 101 (1973).
7. Vos, J. G., and van Genderen, H. Toxicological aspects of immune suppression. In: Pesticides and the Environment: A Continuing Controversy. Symposia Specialists, Miami, 1973, p. 527.
8. Gupta, B., et al. Pathologic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. No. 5: 125 (1973)
9. Zinkl, J. G., et al. Hematologic and clinical chemical effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. No. 5: 111 (1973).
10. Vallejo-Freire, A. A simple technique for repeated collection of blood samples from guinea pigs. Science 114: 524 (1951).
11. Mancini, G., Carbonara, A. O., and Heremans, J. F. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem. 2: 235 (1965).
12. Kliman, B., and Peterson, R. E. Double isotope derivative assay of aldosterone in biological extracts. J. Biol. Chem. 235: 1639 (1960).
13. Miller, R. G. Simultaneous Statistical Inference, McGraw-Hill, New York, 1966.
14. Jonckheere, A. R. A distribution-free K–sample test against ordered alternatives. Biometrika 41: 133 (1954).
15. Jackson, W. T. Regulations of mitosis. III. Cytological effects of 2,4,5-trichlorophenoxyacetic acid and of dioxin contaminants in 2,4,5-T formulations. J. Cell Sci. 10: 15 (1972).
16. Lucier, G. W., et al. Studies on TCDD-induced changes in rat liver microsomal and mitochondrial enzymes. Environ. Health Perspect. No. 5: 199 (1973).
17. Wassermann, M., et al. Effects of organochlorine insecticides on body defense systems. Ann. N. Y. Acad. Sci. 160: 393 (1969).